

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 18, line 18, as follows:

Each subsample of 5,000 seeds is placed in a 1.0 gallon freezer ~~Ziplock~~ Ziploc® bag. Once sealed, this bag is placed inside another ~~Ziplock~~ Ziploc® bag. Both bags are labeled with the sample (batch) number.

Please amend the paragraph beginning at page 19, line 12, as follows:

The required amount of PABST is added to the seed samples in ~~Ziplock~~ Ziploc® bags. The bags are sealed and placed upright in plastic tubs. After the PABST is added to all bags, one ml of the previously made *Xcv* suspension is added to each of the bags in the assay with the exception of the positive control. The bags are closed tightly.

Please amend the paragraph beginning at page 19, line 26, as follows:

The inner bag of seed from the ~~Ziplock~~ Ziploc® bags is removed and placed into funnels. Two or three small vertical slits are cut into one of the bottom corners and the liquid rinsate is collected by allowing it to drain into a centrifuge bottle (such as a 250 ml centrifuge bottle). The seed is discarded. The centrifuge bottles are balanced for centrifuging. If necessary, a small amount of PABST can be added. Centrifuge the rinsate, such as in a GSA rotor in a Sorvall® RC-5B centrifuge, for about 5 minutes at 2500 rpm to pellet the soil and debris. Transfer the supernatant into a clean centrifuge bottle, such as a 250 ml centrifuge bottle. Decant slowly so that if the pellet is soft pieces of the pellet will not accompany the supernatant. If necessary, leave a few milliliters of solution in the bottle to avoid particulates. Centrifuge the filtrate, such as in a GSA rotor in a ~~Sorval~~ Sorvall® RC-5B centrifuge[[]], for about 15 minutes at 5,600 rpm to pellet the bacteria. Discard the supernatant carefully and preserve the pellet. If necessary, leave a few milliliters of solution in a bottle to avoid discarding the soft pellet pieces. About 0.5 grams of polyvinylpyrrolidone (PVPP; see protocol in Example 2d) is added to each centrifuge

bottle. Add the contents of a 5 ml tube of Phos-Tween into the centrifuge bottle and resuspend the pellet. This suspension is incubated at room temperature for about 30 minutes. After about 30 minutes, the suspension is poured through a Miracloth® (Calbiochem, San Diego, California) and collected in a centrifuge tube. The resulting liquid fraction is then centrifuged for about 15 minutes at 7,000 rpm using a SA600 rotor to pellet the bacterial cells. Discard the supernatant. Resuspend the pellet in about 1.3 mls of Phos-Tween, although less can be used if liquid is retained in the tube. This final suspension is now ready for DNA extraction.

Please amend the paragraph beginning at page 20, line 21, as follows:

The final suspension from Example 2a is centrifuged using an Eppendorf® 5415C centrifuge and rotor at about 16,000 g for about 2 minutes. The supernatant is discarded and the pellet resuspended in about 582 µl of TE buffer (See Example 1d for protocol for TE buffer). Add about 15 µl of 20% sodium dodecyl sulfate (SDS) and about 3 µl of Proteinase K (See Example 2d) and the suspension is incubated at 37°C for about 1 to about 1.5 hours. Add about 100 µl of 5M NaCl and 80 µl of hexadecyltrimethylammonium bromide (CTAB) (See Example 2d). The suspension is incubated in a water bath at 65°C for about 15 minutes. Add about 770 µl of chloroform-isoamyl alcohol (24:1) (See Example 2d). The suspension is mixed for about 15 minutes then centrifuged for about 5 minutes at about 16,000 g using an Eppendorf® 5417C centrifuge and rotor. The aqueous supernatant is discarded and placed into a 2 ml tube. About 500 µl of phenol/chloroform/isoamyl alcohol (25:24:1) is added. The suspension is mixed for about 15 minutes and then centrifuged for about 15 minutes at about 16,000 g using [[a]] an Eppendorf® 5417C centrifuge and rotor. About 550 µl of supernatant is transferred to a 1.5 ml tube. About 550 µl of cold isopropyl alcohol is added. The suspension is mixed and stored for at least about four (4) hours at about -80°C.

Please amend the paragraph beginning at page 21, line 9, as follows:

The samples are removed from storage, thawed and centrifuged for about 30 minutes at about 16,000 g using [[a]] an Eppendorf® 5417C centrifuge and rotor. Discard the supernatant

and wash the pellet with about 500 μ l of 70% ethanol. Centrifuge the sample for about 10 minutes at about 16,000 g using an Eppendorf® 5417C centrifuge and rotor. Remove the supernatant and dry the pellet in a vacuum dessicator for about 10 minutes. Redissolve the pellet in about 100 μ l of TE buffer and incubate the samples in a 65°C water bath. Vortex and mix the samples well. Make a 1:50 dilution of the resuspended DNA in water and run about 50 μ l through a BioRad Microspin® column.

Please amend the paragraph beginning at page 21, line 19, as follows:

Load 5 μ l of the DNA suspension from Example 2b into each of the two tubes of PCR reaction mixture. Each reaction well contains: 5 μ l of 10X PCR buffer (Perkin Elmer), 8 μ l of 1.25 mM dNTPs, 3.3 μ l of $MgCl_2$, 1 μ l at 5 pmol/ μ l of each of SEQ ID NO:1 and SEQ ID NO:2, 0.25 of *Taq* polymerase, and 26.45 μ l of water. Each sample tube is run in a PCR thermocycler (Perkin Elmer GeneAmp® PCR System 9600 or an Applied Biosystems GeneAmp® PCR System). The amplifications were conducted under the following conditions: initial DNA denaturation for 10 minutes at about 93°C, followed by a cycling step of about 95° for 1 minute, 62.1°C for 30 seconds, 72°C for about 30 seconds. The PCR cycle is repeated for about 35 cycles. After cycling, there is a final extension step of about 5 minutes at 72°C followed by storage of the products at 4°C until they are removed from the PCR machine. To detect a product, 10 μ l of loading dye (~~defined~~ defined below) is added to each reaction well. About 16 μ l of PCR reaction is added to an electrophoresis gel containing 2% Seakem® LE (defined below) agarose in 0.5X TBE buffer (see Example 2d) and electrophoresed[[.]] as described in Maniatis et al, *Molecular Cloning, a Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989). The gel consists of 2% Seakem® LE in TBE buffer (See Example 2d). The gel is run at 80 or 120 volts depending on the size of the gel.

Please amend the paragraph beginning at page 25, line 23, as follows:

- **PVPP:** Polyvinylpolypyrrolidone (Sigma cat. # P-6755) for BFB-PCR test.
This protocol is taken from Holben *et al* (Appl. Environ. Microbiol. 54:703-711 (1988)).
 - 1-- Mix 300.0 gms of PVPP in 4 liters of 3.0 M Hydrochloric acid. The HCl[.] is prepared by diluting concentrated [HCl] HCl (12.1N)
 - 2-- Mix and let sit at room temperature in a fume hood for 12-16 hrs with occasional gentle stirring.
 - 3-- Filter sterilized the suspension through Miracloth® (Calbiochem, San Diego, CA, Calbiochem cat. #475855). Gentle squeezing of the Miracloth® can be done to remove most of the liquid.
Eventually the Miracloth® will tear, be careful not to lose any PVPP.
 - 4-- Resuspend the PVPP in 4.0 liters of 20 mM Potassium Phosphate buffer, pH=7.4. Prepare the buffer by adding 10.89 gms of KH_2PO_4 to a total of 4.0 liters of MQ water. Adjust the pH to 7.4 using 8.0 M KOH (~10 mls).
 - 5-- Soak at room temperature for 1-2 hrs, gently stirring occasionally.
 - 6-- Filter sterilized the suspension through Miracloth® as in step 3.
 - 7-- Repeat steps 4 through 6 until the pH of the suspension reaches 7.0 (4 to 5 times).
 - 8-- Filter sterilized through Miracloth® as in step 3.
 - 9-- Spread the PVPP out into a thin layer on aluminum foil and dry in a flow hood overnight. Material can be stored in a capped jar and is useful for extended periods of time, >1 year.

Please amend the paragraph beginning at page 40, line 2, as follows:

This Example provides a comparison of specificity of two different primer pairs (RST 90/91 and SEQ ID NOS: 1-2). Comparisons were made with isolates of *Acidovorax* spp. and

numerous other bacterial isolates. All DNA was prepared using the 'boil prep' method which consists of making a suspension of the bacteria in 200 ul of water in a 1.5 ml Eppendorf® tube and placing the tubes in a boiling water bath for about 10 minutes. Data is a compilation of two different experiments (PCR #71 and # 169). ND=not done.

Please amend the paragraph beginning at page 41, line 25, as follows:

This Example shows the effects of diluting the extracted DNA prior to running the material through a BioRad Sephadex® column on the ability of the PCR to detect the presence of *Acidovorax avenae* pv *citrulli*. A dilution series (10 fold) was made from an original suspension of *Aac* that was 0.1 OD 620_{nm}. Specific amounts were added to samples of 5,000 Charleston Grey watermelon seed. Further dilutions were plated onto Nutrient Agar and counts were made to determine the amount of cells added to the samples. Values are expressed as Colony Forming Units (CFU)/ml of seedwash suspension. Results are expressed as the number of positive PCR reactions over the total number of reactions.